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COMPUTER-ASSISTED LABELLING IN MUTAGENICITY TESTING THE 1/1  
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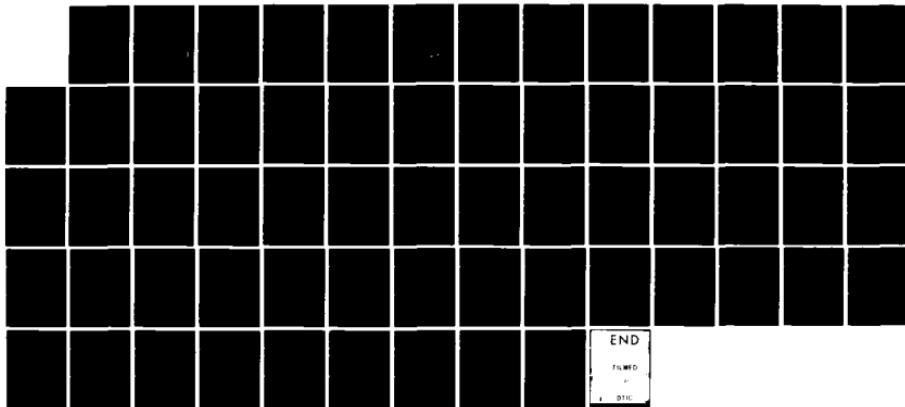
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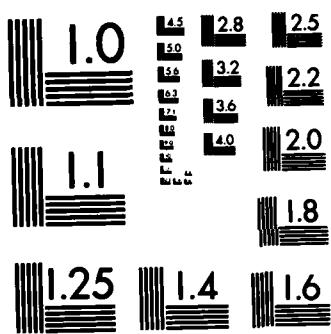
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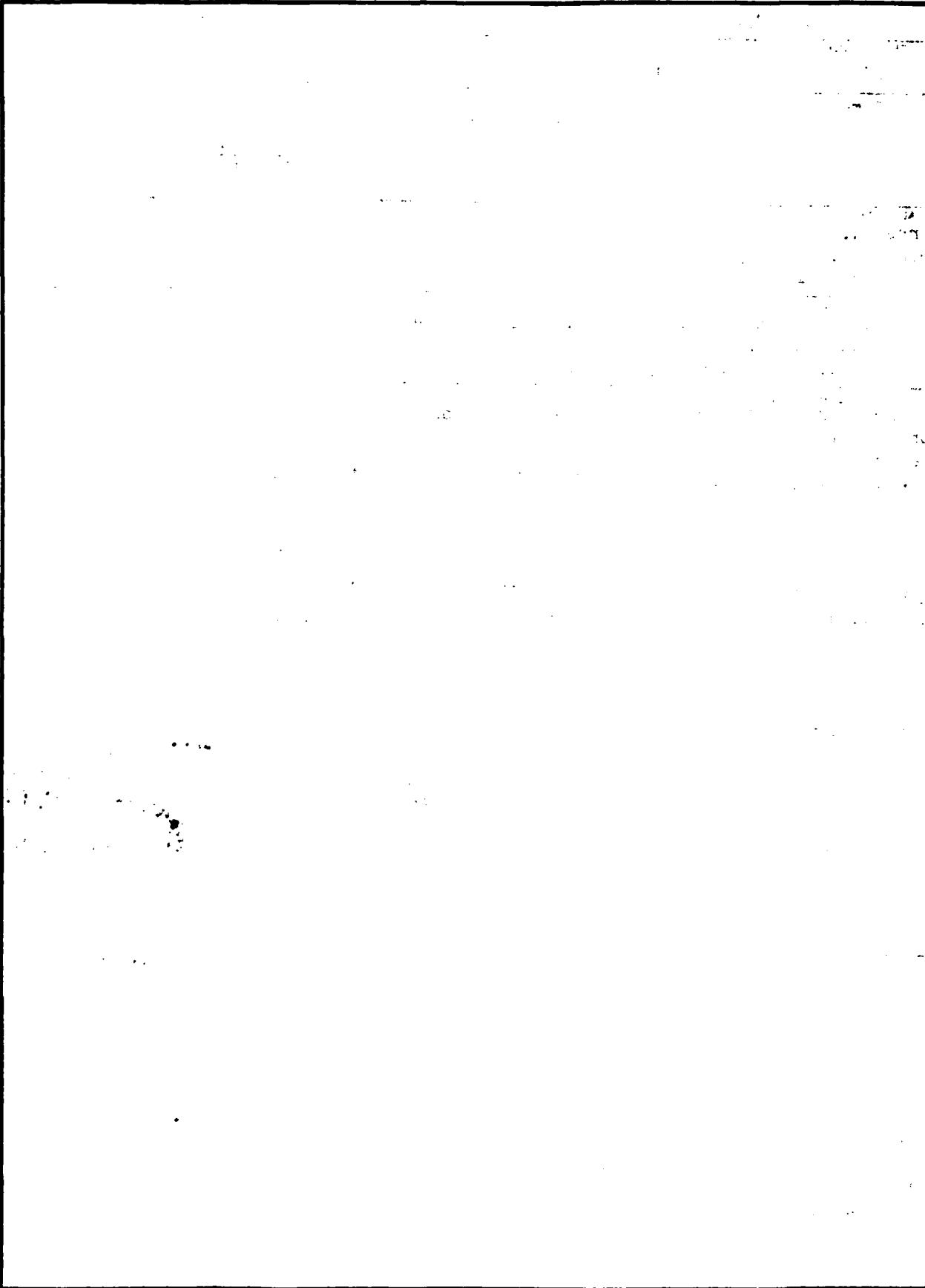
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**TECHNICAL NOTE NO. 82-40TN**

**COMPUTER-ASSISTED LABELLING IN MUTAGENICITY TESTING  
The Ames Salmonella/Mammalian Microsome Mutagenicity Assay**

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and  
TOXICOLOGY GROUP,  
DIVISION OF RESEARCH SUPPORT**

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Computer-assisted Labelling in Mutagenicity Testing:  
The Ames Salmonella/Mammalian Microsome Mutagenicity Assay--Jederberg et al

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## PREFACE

This is the first in a series of Technical Notes on the utilization of the computer facilities at Letterman Army Institute of Research to assist in mutagenicity testing as part of the institute's toxicology program. These reports will detail the use of the computer in labelling articles, recording, storing, and retrieving data, and data analysis. Both the Salmonella/Mammalian microsome (Ames) and the Drosophila melanogaster sex-linked recessive lethal mutagenicity assays will be covered. These reports are not intended to cover in detail the rationale or the technique involved in mutagenicity testing by these assays.

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**COMPUTER-ASSISTED LABELLING IN MUTAGENICITY TESTING:**  
The Ames Salmonella/Mammalian Microsome Mutagenicity Assay--Jederberg, Sauers, and Pulliam

The Ames Salmonella/Mammalian Microsome Mutagenicity Assay is one of a standard bank of tests used for the detection of mutagenic agents (1). It involves the use of several genetically altered strains of Salmonella typhimurium - each with a specific mutation in the histidine operon. The test is based on a reverse mutation to a wild-type, prototrophic phenotype induced by the test compound. In addition to the reversion, activation by microsomal enzymes is also used to determine if any potential promutagens exist.

This type of assay requires the labelling of agar plates with information concerning the dilution of the test substance, the particular bacterial mutant, the presence or absence of microsomal enzymes, and replicates. Since all assay components are added to a tube of top agar prior to plating, a corresponding number of labels must also be generated. This requires the production of approximately 400 labels per test substance and approximately 300 labels for controls per assay. These items are currently hand-labelled in most laboratories. We have designed and implemented a FORTRAN V program (with subroutines) for the rapid generation of the large number of unique labels. The use of this system has greatly reduced the time spent identifying material, guaranteed accurate correspondence between the top agar tube and the target plate, and insured continuity from the initiation to the termination of the assay.

This technical note describes the programs used for labelling the numerous articles used in performing the assay. For a complete discussion of the nature of the required materials for the assay, the reader is referred to reference 1.

**PROGRAM DESCRIPTION**

The main program (Appendix A) calls a series of subroutines to generate sterility, quality, positive and negative control, test compound, and dilutional control labels (Appendix B through K). The labels are generated in the sequence that they will be used in setting up the assay.

Sample runs appear in Figures 1 through 11 (Appendix L). As the program prepares the labels, it displays a message (Figure 1). Up to ten test compounds are allowed and each is designated with an

alphanumeric field of up to ten characters in length (AN/10). Up to ten dilutions of each test compound are allowed. (In the figure, the underlined characters are entered at the time of program execution by the user.)

The text for sterility control labels appears in Figures 2 through 5. Sterility controls include (a) minimal glucose agar (MGA) and the nutrient broth (Figure 2), without the presence of mammalian microsome solution (S-9), (b) the top agar and S-9 mix (Figure 3), (c) the diluent and histidine-biotin mixture (Figure 4), and each of the bacterial cultures (Figure 5).

A check is made on the characteristic nutrient requirements of the strains used (Figure 6).

Quality controls check the characteristic responses of the mutant strains to chemical and light stimuli (Figure 7).

The response of each strain to known positive mutagens is validated (Figure 8).

The test strains are grown, both, with no S-9 and with S-9 to check the spontaneous mutation rates (Figure 9).

At the beginning of each test compound, the diluent used for that compound is checked, both, with and without S-9 (Figure 10).

Each test compound is checked with each tester strain of microorganism in the presence and absence of S-9 at each dilution of the test compound (samples represented in Figure 11).

At the end of the run the negative and sterility controls are run again.

After the text for all the labels has been generated and stored in the file labelled "DLABELS" the user is so notified and may print the labels out on an appropriate device (usually a printer with tractor feed). The test is formatted so that the labels should be printed on continuous-feed single-width stock material. These may be obtained from most distributors of computer supplies and office forms. The labels are standard 1 x 3.5 inches.

#### DISCUSSION

By utilizing the program and subroutines presented in this report, we have realized a significant saving in time for the preparation of materials for the Salmonella/Mammalian microsome mutagenicity assay. The labels have also been of great help in maintaining order and

uniformity while running the assay. The labelling has been beneficial in maintaining conformity with the rigid requirements of the Food and Drug Administration's Good Laboratory Practices Act (2).

**CONCLUSION**

**None**

**RECOMMENDATION**

**None**

REFERENCES

1. AMES, B.N., J. McCANN and E. YAMASAKI. Methods for detecting carcinogens and mutagens with the salmonella/mammalian microsome mutagenicity assay. *Mut Res* 31:347-364, 1975.
2. FOOD AND DRUG ADMINISTRATION. Good Laboratory Practices Regulations. *Federal Register* 43(163):37336-37403, 1978.

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## APPENDICES

Appendix A.  
AMESLABELS.FR

C --- PROGRAM NAME: AMESLABELS.FR  
C --- WRITTEN BY: WARREN W JEDERBERG  
C --- PURPOSE: TO PREPARE LABELS FOR THE AMES  
C --- MUTAGENICITY ASSAY.  
C --- SUBROUTINES:  
C --- SCLW.FR-STERILITY CONTROL LABELS WRITTEN  
C --- SUPPORTED BY SLAB.FR  
C --- QCLW.FR-QUALITY CONTROL LABELS WRITTEN  
C --- PCLW.FR-POSITIVE CONTROL LABELS WRITTEN  
C --- NCLW.FR-NEGATIVE CONTROL LABELS WRITTEN  
C --- ATLW.FR-TEST COMPOUND LABELS WRITTEN  
C --- KLBW.FR SUPPORTS ATLW.FR  
C --- NLBW.FR SUPPORTS ATLW.FR  
C --- DLCLW.FR SUPPORTS ATLW.FR  
C --- TLCW.FR-END RUN STERILITY CONTROL LABELS WRITTEN  
C --- SUPPORTED BY SLAB.FR  
C ---

DELETE "ALABELS"  
OPEN 1, "ALABELS", ATT = "SOP"  
TYPE  
TYPE " READY TO MAKE AMESLABELS:"  
TYPE " WAIT ....."  
DIMENSION LT(5)  
LT(1) = "ST"  
LT(2) = "AR"  
LT(3) = "T "  
LT(4) = "RU"  
LT(5) = "N "

CALL SCLW(LT)  
CALL QCLW

CALL PCLW  
CALL NCLW(LT)  
CALL ATLW

LT(1) = "BN"  
LT(2) = "D "  
LT(3) = "RU"  
LT(4) = "N "  
LT(5) = " "

CALL NCLW(LT)  
CALL TLCW(LT)

**Appendix A.**  
**(CONTINUED)**

TYPE " ALL LABELS ARE READY IN 'ALABELS'"  
CLOSE !  
END

Appendix B.  
SCLW.FR

```
C --- PROGRAM NAME: SCLW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE WRITES THE STERILITY CONTROL
C --- LABELS FOR THE AMES MUTAGENICITY ASSAY. SUPPORTED BY SLAB.FR.
C ---
C --- SUBROUTINE SCLW (LT)
C --- DIMENSION NBUG(2), LT(5)

DO 90 I=1,2
WRITE (1,4)
4 FORMAT (2X,"STERILITY CONTROL",/,2X,"MGA PLATE",/,2X,
1"ADD WILD TYPE",/,2X,"DO NOT ADD S-9",/,2X,"24 HRS",/)
90 CONTINUE

DO 100 I=1,2
WRITE (1,5)
5 FORMAT (2X,"STERILITY CONTROL",/,2X,"MGA PLATE",/,2X,
1"ADD NO ORGANISM",/,2X,"DO NOT ADD S-9",/,2X,"24 HRS",/)
100 CONTINUE

DO 101 I=1,2
WRITE (1,6)
6 FORMAT (2X,"STERILITY CONTROL",/,2X,"NUTRIENT BROTH",/,2X,
1"ADD NO ORGANISM",/,2X,"DO NOT ADD S-9",/)
101 CONTINUE

DO 10 I=1,4
WRITE (1,1) LT
1 FORMAT (2X,"STERILITY CONTROL",/,2X,"TOP AGAR",/,
12X,"ADD NO ORGANISMS",/,2X,"DO NOT ADD S-9",/,2X,5A2,/)
10 CONTINUE

DO 20 I=1,4
WRITE (1,2) LT
2 FORMAT (2X,"STERILITY CONTROL",/,2X,"TOP AGAR",/,
12X,"ADD NO ORGANISMS",/,2X," ADD S-9",/,2X,5A2,/)
20 CONTINUE

DO 30 I=1,4
WRITE (1,3) LT
3 FORMAT (2X,"STERILITY CONTROL",/,2X,"DILUENT",/,
12X,"ADD NO ORGANISMS",/,2X,"DO NOT ADD S-9",/,2X,5A2,/)
30 CONTINUE
```

Appendix B.  
(CONTINUED)

```
DO 102 I=1,2
WRITE (1,7) LT
7 FORMAT (2X,"STERILITY CONTROL",/,2X,"HIS-BIO MIX",/,2X,
  !"ADD NO ORGANISMS",/,2X,"DO NOT ADD S-9",/,2X,5A2,/)
102 CONTINUE
```

C TA 98

```
NBUG(1) = " "
NBUG(2) = "98"
CALL SLAB(NBUG,LT)
```

C TA 100

```
NBUG(1) = " "
NBUG(2) = "00"
CALL SLAB(NBUG,LT)
```

C TA 1535

```
NBUG(1) = "15"
NBUG(2) = "35"
CALL SLAB(NBUG,LT)
```

C TA 1537

```
NBUG(1) = " "
NBUG(2) = "37"
CALL SLAB(NBUG,LT)
```

C TA 1538

```
NBUG(1) = " "
NBUG(2) = "38"
CALL SLAB(NBUG,LT)
```

C --- READY MESSAGE

```
TYPE " STERILITY CONTROL LABELS ARE READY IN 'ALABELS'"
RETURN
END
```

Appendix C.  
QCLW.FR

```
C --- PROGRAM NAME: QCLW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE WRITES THE QUALITY CONTROL
C --- LABELS FOR THE AMES MUTAGENICITY ASSAY.
C ---
C --- SUBROUTINE QCLW
C --- DIMENSION NBUG(2)

      WRITE (1,1)
1   FORMAT(2X,"0.1 M HIS & 0.5 BIOTIN",/,
     12X,"TA: 1535, 1537, 1538",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",//)

      WRITE (1,2)
2   FORMAT(2X,"0.1 M HIS & 0.5 BIOTIN",/,
     12X,"TA: 98, 100, WT",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",//)

      WRITE (1,3)
3   FORMAT(2X,"0.5 BIOTIN ONLY",/,
     12X,"TA: 1535, 1537, 1538",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",//)

      WRITE (1,4)
4   FORMAT(2X,"0.5 BIOTIN ONLY",/,
     12X,"TA: 98, 100, WT",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",//)

      WRITE (1,5)
5   FORMAT(2X,"QUALITY CONTROL",/,2X,"UV LIGHT: 6 SECONDS",/,
     12X,"TA: 1535, 1537, 1538",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",/)

      WRITE (1,6)
6   FORMAT(2X,"QUALITY CONTROL",/,2X,"UV LIGHT: 8 SECONDS",/,
     12X,"TA: 98, 100, WT",/,
     22X,"DO NOT ADD S-9",/,2X,"DO NOT ADD TOP AGAR",/)

      NBUG(1) = " "
      NBUG(2) = "qz"

      WRITE (1,7) NBUG
      WRITE (1,7) NBUG
```

Appendix C.  
(CONTINUED)

```
NBUG(1) = " 1"
NBUG(2) = "00"

WRITE (1,7) NBUG
WRITE (1,7) NBUG

NBUG(1) = "15"
NBUG(2) = "35"

WRITE (1,7) NBUG
WRITE (1,7) NBUG

NBUG(2) = "37"

WRITE (1,7) NBUG
WRITE (1,7) NBUG

NBUG(2) = "38"

WRITE (1,7) NBUG
WRITE (1,7) NBUG

7 FORMAT(2X,"QUALITY CONTROL",/,2X,"CRYSTAL VIOLET",/,
12X,"TA",1X,2A2,/,2X,"ADD TOP AGAR",//)

NBUG(1) = " "
NBUG(2) = "98"

WRITE (1,8) NBUG

NBUG(1) = " 1"
NBUG(2) = "00"

WRITE(1,8) NBUG

NBUG(1) = "15"
NBUG(2) = "37"

WRITE (1,8) NBUG

8 FORMAT (2X,"QUALITY CONTROL",/,2X,"AMPICILLIN",/,
12X,"TA",1X,2A2,/,2X,"DO NOT ADD TOP AGAR",//)

C --- END MESSAGE
TYPE " QUALITY CONTROL LABELS ARE READY IN 'ALABELS'"
```

RETURN  
END

Appendix D.  
PCLW.FR

```
C --- PROGRAM NAME:          PCLW.FR
C --- WRITTEN BY:           WARREN W. JEDERBERG
C --- PURPOSE:              THIS SUBROUTINE WRITES THE POSITIVE
C ---                      CONTROL LABELS FOR THE AMES MUTAGENICITY ASSAY.
C ---
C --- SUBROUTINE PCLW
DIMENSION KOMP(5),NBUG(2)

C      BP 2 UG
NBUG(1) = " "
NBUG(2) = "98"

KOMP(1) = "EP"
KOMP(2) = " "
KOMP(3) = "2 "
KOMP(4) = "UG"
KOMP(5) = " "

DO 10 I=1,6
WRITE (1,1) NBUG,KOMP
1  FORMAT(2X,"POSITIVE CONTROL",/,2X,"TA",1X,2A2,/,
12X,5A2,/,2X," ADD S-9",//)
10 CONTINUE

NBUG(1) = "1"
NBUG(2) = "00"

DO 20 I=1,6
WRITE (1,1) NBUG,KOMP
20 CONTINUE

NBUG(1) = "15"
NBUG(2) = "37"

DO 30 I=1,6
WRITE (1,1) NBUG, KOMP
30 CONTINUE

NBUG(2) = "58"

DO 40 I=1,6
WRITE (1,1) NBUG, KOMP
40 CONTINUE
```

Appendix D.  
(CONTINUED)

```
C      DMBA 20 UG
      KOMP(1) = "DM"
      KOMP(2) = "BA"
      KOMP(3) = " 2"
      KOMP(4) = "O "
      KOMP(5) = "UG"

      NBUG(1) = "  "
      NBUG(2) = "98"

      DO 50 I=1,6
      WRITE (1,1) NBUG,KOMP
50    CONTINUE

      NBUG(1) = " 1"
      NBUG(2) = "OO"

      DO 60 I=1,6
      WRITE (1,1) NBUG,KOMP
60    CONTINUE

      NBUG(1) = "15"
      NBUG(2) = "37"

      DO 70 I=1,6
      WRITE (1,1) NBUG,KOMP
70    CONTINUE

      NBUG(2) = "38"

      DO 80 I=1,6
      WRITE (1,1) NBUG,KOMP
80    CONTINUE

C      AF 2 UG
      KOMP(1) = "AF"
      KOMP(2) = " 2"
      KOMP(3) = " U"
      KOMP(4) = "G "
      KOMP(5) = "  "

      NBUG(1) = "  "
      NBUG(2) = "98"

      DO 90 I=1,6
      WRITE (1,1) NBUG, KOMP
90    CONTINUE
```

Appendix D.  
(CONTINUED)

```
NBUG(1) = "1"
NBUG(2) = "00"

DO 100 I=1,6
WRITE (1,1) NBUG, KOMP
100 CONTINUE

NBUG(1) = "15"
NBUG(2) = "38"

DO 110 I=1,6
WRITE (1,1) NBUG, KOMP
110 CONTINUE

C      MNNG 2 UG
KOMP(1) = "MN"
KOMP(2) = "NG"
KOMP(3) = " 2"
KOMP(4) = " U"
KOMP(5) = "G "

NBUG(1) = "1"
NBUG(2) = "00"

DO 120 I=1,6
WRITE (1,2) NBUG, KOMP
2      FORMAT (2X,"POSITIVE CONTROL",/,2X,"TA",1X,2A2,/,
12X,5A2,/2X,"DO NOT ADD S-9",//)
120 CONTINUE

NBUG(1) = "15"
NBUG(2) = "35"

KOMP(4) = "O "
KOMP(5) = "UG"

DO 130 I=1,6
WRITE (1,2) NBUG, KOMP
130 CONTINUE

C --- READY MESSAGE
TYPE " POSITIVE CONTROL LABELS ARE READY IN 'ALABELS'"
RETURN
END
```

Appendix E.  
NCLW.FR

C --- PROGRAM NAME: NCLW.FR  
C --- WRITTEN BY: WARREN W JEDERBERG  
C --- PURPOSE: THIS SUBROUTINE PREPARES THE NEGATIVE  
C --- CONTROL LABELS FOR THE AMES MUTAGENICITY ASSAY.  
C ---  
SUBROUTINE NCLW(LT)  
DIMENSION NBUG(2), LT(5)

C NO S-9

NBUG(1) = " "  
NBUG(2) = "98"

DO 10 I=1,6  
WRITE (1,1) NBUG, LT  
1 FORMAT (2X,"NEGATIVE CONTROL",/,2X,"TA",1X,2A2,/,  
1 2X,"DO NOT ADD S-9",/,2X,5A2,//)  
10 CONTINUE

NBUG(1) = " 1"  
NBUG(2) = "00"

DO 20 I=1,6  
WRITE (1,1) NBUG, LT  
20 CONTINUE

NBUG(1) = "15"  
NBUG(2) = "35"

DO 30 I=1,6  
WRITE (1,1) NBUG, LT  
30 CONTINUE

NBUG(2) = "37"

DO 40 I=1,6  
WRITE (1,1) NBUG, LT  
40 CONTINUE

NBUG(2) = "38"

DO 50 I=1,6  
WRITE (1,1) NBUG, LT  
50 CONTINUE

Appendix E.  
(CONTINUED)

C WITH S-?

```
NBUG(1) = " "
NBUG(2) = "98"

DO 60 I=1,6
WRITE (1,2) NBUG,LT
2 FORMAT(2X,"NEGATIVE CONTROL",/,2X,"TA",1X,2A2,/
12X," ADD S-9",/,2X,5A2,//)
60 CONTINUE

NBUG(1) = " 1"
NBUG(2) = "00"

DO 70 I=1,6
WRITE (1,2) NBUG,LT
70 CONTINUE

NBUG(1) = "15"
NBUG(2) = "35"

DO 80 I=1,6
WRITE (1,2) NBUG,LT
80 CONTINUE

NBUG(2) = "37"

DO 90 I=1,6
WRITE (1,2) NBUG,LT
90 CONTINUE

NBUG(2) = "58"
DO 100 I=1,6
WRITE (1,2) NBUG,LT
100 CONTINUE

C --- READY MESSAGE
TYPE " NEGATIVE CONTROL LABELS ARE READY IN 'ALABELS' "
RETURN
END
```

Appendix F.  
ATLW.FR

```
C --- PROGRAM NAME: ATLW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE WRITE THE TEST COMPOUND
C --- LABELS FOR THE AMES MUTAGENCITY ASSAY. IT USES THE
C -- SUBROUTINES DLCLW.FR, KLBW.FR, NLBW.FR.
C ---
C --- SUBROUTINE ATLW
C --- DIMENSION NBUG(2), KN(5)
C GET THE NUMBER OF TEST COMPOUNDS
TYPE
TYPE " HOW MANY TEST COMPCUNDS :"
ACCEPT "*",NC

C WRITE LABELS FOR EACH COMPOUND
DO 10 I=1,NC
TYPE
WRITE (10,1) I
1 FORMAT (" WHAT IS COMPOUND #:",1X,12,1X,"(AN/10) ?")
READ (11,2) (KN(IL), IL=1,5)
2 FORMAT (5A2)
C HOW MANY DILUTIONS FOR THIS COMPOUND
20 CONTINUE
TYPE
TYPE " HOW MANY DILUTIONS FOR THIS COMPOUND ?"
ACCEPT "*",N

IF (N.GT.10) GO TO 30

C MAKE DILUTIONAL CONTROL LABELS
CALL DLCLW (KN)

DO 100 J=1,N

C BY BUG - NO S-9
NBUG(1) = " "
NBUG(2) = "98"

CALL KLBW (NBUG,KN,J)

NBUG(1) = " 1"
NBUG(2) = "00"
```

Appendix F.  
(CONTINUED)

CALL KLBW (NBUG,KN,J)

NBUG(1) = "15"

NBUG(2) = "35"

CALL KLBW (NBUG,KN,J)

NBUG(2) = "37"

CALL KLBW (NBUG,KN,J)

NBUG(2) = "38"

CALL KLBW (NBUG,KN,J)

C BY BUG WITH S-9

NBUG(1) = " "

NBUG(2) = "98"

CALL NLBW (NBUG,KN,J)

NBUG(1) = " 1"

NBUG(2) = "00"

CALL NLBW (NBUG,KN,J)

NBUG(1) = "15"

NBUG(2) = "35"

CALL NLBW (NBUG,KN,J)

NBUG(2) = "37"

CALL NLBW (NBUG,KN,J)

NBUG(2) = "38"

CALL NLBW (NBUG,KN,J)

'00 CONTINUE

Appendix F.  
(CONTINUED)

```
      WRITE (11,1)
3   FORMAT (" LABELS FOR TEST COMPOUND #:",1X,12,1X,"ARE READY IN 'ALABELS'")
10  CONTINUE
    RETURN
C   ERROR NOTE
30  CONTINUE
    TYPE
    TYPE "** ERROR ** ERROR **"
    TYPE "NO MORE THAN 10 ALLOWED"
    GO TO 20
    END
```

Appendix G.  
DLCLW.FR

```
C --- PROGRAM NAME: DLCLW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE PREPARES THE DILUTIONAL
C --- CONTROL LABELS IN SUPPORT OF ATLW.FR FOR THE
C --- AMES MUTAGENICITY ASSAY.
C ---
C --- SUBROUTINE DLCLW (KN)
C --- DIMENSION KN(5)

C      NO S-9
C      DO 30 I=1,4
C      WRITE (1,1) KN
1     FORMAT (2X,"DILUTIONAL CONTROL",/,2X,"COMPOUND:",'X,5A2,/,
12X,"ADD NO ORGANISMS",/,
12X,"DO NOT ADD S-9",//)
30    CONTINUE

C      WITH S-9
C      DO 50 I=1,4
C      WRITE (1,2) KN
2     FORMAT (2X,"DILUTIONAL CONTROL",/,2X,"COMPOUND:",'X,5A2,/,
12X,"ADD NO ORGANISMS",/,
12X,2X," ADD S-9",//)
50    CONTINUE

C --- READY MESSAGE
TYPE
TYPE " DILUTIONAL CONTROL LABELS ARE READY IN 'ALABELS'""
RETURN
END
```

Appendix H.  
KLBW.FR

```
C --- PROGRAM NAME: KLBW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE SUPPORTS THE SUBROUTINE
C --- ATLW.FR IN WRITING THE TEST COMPOUND LABELS FOR THE
C --- Ames mutagenicity assay.
C ---
C --- SUBROUTINE KLBW(NBUG,KN,J)
C --- DIMENSION NBUG(2),KN(5)

C      NO S-9
DO 20 JK=1,6
      WRITE (1,1) NBUG, (KN(I) I=1,5),J
1      FORMAT (2X,"TEST SUBSTANCE",/,2X,"TA",1X,2A2,/,
12X,"COMPOUND:",1X,5A2,/,2X,"DILUTION:",1X,I2./,
22X,"DO NOT ADD S-9",/)
20    CONTINUE
      RETURN
      END
```

Appendix I.  
NLBW.FR

```
C --- PROGRAM NAME: NLBW.FR
C --- WRITTEN BY: WARREN W JEDERBERG
C --- PURPOSE: THIS SUBROUTINE SUPPORTS THE SUBROUTINE
C --- ATLW.FR IN WRITING THE TEST COMPOUND LABELS FOR THE
C --- AMES MUTAGENICITY ASSAY.
C ---
C      SUBROUTINE NLBW (NBUG,KN,J)
C      DIMENSION NBUG(2),KN(5)

C      WITH S-9
DO 20 JK=1,6
      WRITE (1,1) NBUG,(KN(I) I=1,5),J
1      FORMAT (2X,"TEST SUBSTANCE",/,2X,"TA",1X,2A2,/,
12X,"COMPOUND:",1X,5A2,/,2X,"DILUTION:",1X,I2,/,
22X," ADD S-9",/)
20      CONTINUE
      RETURN
      END
```

Appendix J.  
TLCW.FR

C --- PROGRAM NAME: TLCW.FR  
C --- WRITTEN BY: WARREN W JEDERBERG  
C --- PURPOSE: THIS SUBROUTINE WRITES THE STERILITY CONTROL  
C --- LABELS FOR THE AMES MUTAGENICITY ASSAY. SUPPORTED BY SLAB.FR.  
C --- (END RUN)  
C ---  
C       SUBROUTINE TLCW (LT)  
DIMENSION NBUG(2), LT(5)  
  
DO 10 I=1,4  
WRITE (1,1) LT  
1 FORMAT (2X,"STERILITY CONTROL",/,2X,"TOP AGAR",/,  
12X,"ADD NO ORGANISMS",/,2X,"DO NOT ADD S-9",/,2X,5A2,/)  
10 CONTINUE  
  
DO 20 I=1,4  
WRITE (1,2) LT  
2 FORMAT (2X,"STERILITY CONTROL",/,2X,"TOP AGAR",/,  
12X,"ADD NO ORGANISMS",/,2X,"ADD S-9",/,2X,5A2,/)  
20 CONTINUE  
  
DO 30 I=1,2  
WRITE (1,3) LT  
3 FORMAT (2X,"STERILITY CONTROL",/,2X,"HIS-BIO MIX",/,2X,  
1"ADD NO ORGANISMS",/,2X,"DO NOT ADD S-9",/,2X,5A2,/)  
30 CONTINUE  
  
C       TA 98  
  
NBUG(1) = " "  
NBUG(2) = "98"  
CALL SLAB(NBUG,LT)  
  
C       TA 100  
  
NBUG(1) = " 1"  
NBUG(2) = "00"  
CALL SLAB(NBUG,LT)  
  
C       TA 1535  
  
NBUG(1) = "15"  
NBUG(2) = "35"  
CALL SLAB(NBUG,LT)

Appendix J.  
(CONTINUED)

C TA 1557

NBUG(2) = "57"  
CALL SLAB(NBUG,LT)

C TA 1558

NBUG(2) = "58"  
CALL SLAB(NBUG,LT)

C --- READY MESSAGE

TYPE " STERILITY CONTROL LABELS ARE READY IN 'ALABELS'"

TYPE " (END PUN)"

RETURN

END

Appendix K.  
SLAB.FR

C --- PROGRAM NAME: SLAB.FR  
C --- WRITTEN BY: WARREN W JEDERBERG  
C --- PURPOSE: THIS SUBROUTINE IS SUPPORTING THE  
C --- WRITING OF STERILITY CONTROL LABELS FOR THE AMES  
C --- MUTAGENICITY ASSAY. USED BY SCLW.FR.

```
SUBROUTINE SLAB (NBUG,LT)
DIMENSION NBUG(2), LT(5)
DO 10 I=1,4
  WRITE (1,1) NBUG,LT
1 FORMAT (2X,"STERILITY CONTROL",/,2X,"TA",1X,2A2,/,
12X,"ADD NO TOP AGAR",/,2X,"DO NOT ADD S-1",/,2X,5A2,/)
10 CONTINUE
RETURN
END
```

## LIST OF FIGURES

- Figure 1.** Sample Run of AMESLABELS.FR.....
- Figure 2.** Sample Label Texts for Sterility Controls  
MGA and Nutrient Broth.....
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- Figure 8.** Sample Label Texts for the Positive Control Responses  
of Microbial Strains (with and without S-9).....
- Figure 9.** Sample Label Texts for the Negative Control Responses  
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- Figure 10.** Sample Label Texts for Dilutional Control  
Check for a Test Compound.....
- Figure 11.** Sample Label Texts for a Test Compound  
(with and without S-9).....

APPENDIX L (Figures)

Figure 1. Sample run of AMESLABELS.FR

) X AMESLABELS

READY TO MAKE AMESLABELS:

WAIT .....

STERILITY CONTROL LABELS ARE READY IN 'ALABELS'

QUALITY CONTROL LABELS ARE READY IN 'ALABELS'

POSITIVE CONTROL LABELS ARE READY IN 'ALABELS'

NEGATIVE CONTROL LABELS ARE READY IN 'ALABELS'

HOW MANY TEST COMPOUNDS ?

\*2

WHAT IS COMPOUND #: 1 (AN/10) ?

CMPND #1

HOW MANY DILUTIONS FOR THIS COMPOUND ?

\*2

DILUTIONAL CONTROL LABELS ARE READY IN 'ALABELS'

LABELS FOR TEST COMPOUND #: 1 ARE READY IN 'ALABELS'

WHAT IS COMPOUND #: 2 (AN/10) ?

CMPND #2

HOW MANY DILUTIONS FOR THIS COMPOUND ?

\*2

DILUTIONAL CONTROL LABELS ARE READY IN 'ALABELS'

LABELS FOR TEST COMPOUND #: 2 ARE READY IN 'ALABELS'

NEGATIVE CONTROL LABELS ARE READY IN 'ALABELS'

STERILITY CONTROL LABELS ARE READY IN 'ALABELS'

(END RUN)

ALL LABELS ARE READY IN 'ALABELS'

)

**Figure 2. Sample Label Texts for Sterility Controls  
(MGA and Nutrient Broth)**

STERILITY CONTROL  
MGA PLATE  
ADD WILD TYPE  
DO NOT ADD S-9  
24 HRS

STERILITY CONTROL  
MGA PLATE  
ADD WILD TYPE  
DO NOT ADD S-9  
24 HRS

STERILITY CONTROL  
MGA PLATE  
ADD NO ORGANISM  
DO NOT ADD S-9  
24 HRS

STERILITY CONTROL  
MGA PLATE  
ADD NO ORGANISM  
DO NOT ADD S-9  
24 HRS

STERILITY CONTROL  
NUTRIENT BROTH  
ADD NO ORGANISM  
DO NOT ADD S-9

STERILITY CONTROL  
NUTRIENT BROTH  
ADD NO ORGANISM  
DO NOT ADD S-9

**Figure 3. Sample Label Texts for Sterility Controls  
(Top Agar)**

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
ADD S-9  
START RUN

STERILITY CONTROL  
TOP AGAR  
ADD NO ORGANISMS  
ADD S-9  
START RUN

**Figure 4. Sample Label Texts for Sterility Controls  
(Diluent and His-Bio Mix)**

STERILITY CONTROL  
DILUENT  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
DILUENT  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
DILUENT  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
DILUENT  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
HIS-BIO MIX  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
HIS-BIO MIX  
ADD NO ORGANISMS  
DO NOT ADD S-9  
START RUN

**Figure 5. Sample Label Texts for Sterility Controls  
(Microbial Strain Checks)**

STERILITY CONTROL  
TA 20  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 20  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 20  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 20  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 100  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 100  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 100  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 100  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

**Figure 5. Sample Label Texts for Sterility Controls  
(Microbial Strain Checks)**

**CONTINUED**

STERILITY CONTROL  
TA 1535  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1535  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1535  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1535  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1537  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1537  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1537  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1537  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

**Figure 5. Sample Label Texts for Sterility Controls  
(Microbial Strain Checks)**  
CONTINUED

STERILITY CONTROL  
TA 1538  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1538  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1538  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

STERILITY CONTROL  
TA 1538  
ADD NO TOP AGAR  
DO NOT ADD S-9  
START RUN

**Figure 6. Sample Label Texts for Microbial Nutrient Requirements**

0.1 M HIS & 0.5 BIOTIN  
TA: 1535, 1537, 1538  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

0.1 M HIS & 0.5 BIOTIN  
TA: 98, 100, WT  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

0.5 BIOTIN ONLY  
TA: 1535, 1537, 1538  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

0.5 BIOTIN ONLY  
TA: 98, 100, WT  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

**Figure 7. Sample Label Texts for Microbial Response  
to Environmental Stimuli**

QUALITY CONTROL  
UV LIGHT: 6 SECONDS  
TA: 1525, 1537, 1539  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

QUALITY CONTROL  
UV LIGHT: 9 SECONDS  
TA: 98, 100, WT  
DO NOT ADD S-9  
DO NOT ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 98  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 98  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 100  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 100  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1525  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1525  
ADD TOP AGAR

Figure 7. Sample Label Texts for Microbial Response  
to Environmental Stimuli  
CONTINUED

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1537  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1537  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1538  
ADD TOP AGAR

QUALITY CONTROL  
CRYSTAL VIOLET  
TA 1538  
ADD TOP AGAR

QUALITY CONTROL  
AMPICILLIN  
TA 98  
DO NOT ADD TOP AGAR

QUALITY CONTROL  
AMPICILLIN  
TA 100  
DO NOT ADD TOP AGAR

QUALITY CONTROL  
AMPICILLIN  
TA 1537  
DO NOT ADD TOP AGAR

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9)**

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 100  
BP 2 UG  
ADD S-9

POSITIVE CONTROL

TA 100  
BP 2 UG  
ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

**POSITIVE CONTROL**

TA 100

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 100

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 100

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 100

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 1537

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 1537

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 1537

BP 2 UG

ADD S-9

**POSITIVE CONTROL**

TA 1537

BP 2 UG

ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL

TA 1537

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1537

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1539

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1539

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1539

BP 2 UG

ADD S-9

POSITIVE CONTROL

TA 1539

BP 2 UG

ADD S-9

Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 98  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 100  
DMBA 20 UG  
ADD S-9

POSITIVE CONTROL

TA 100  
DMBA 20 UG  
ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL.

TA 100  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 100  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 100  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 100  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL.

TA 1537  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 1537  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 1537  
DMBA 20 µG  
ADD S-9

POSITIVE CONTROL

TA 1537  
DMBA 20 µG  
ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL.

TA 1537

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1537

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

POSITIVE CONTROL

TA 1538

DMBA 20 UG

ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 98  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 100  
AF 2 UC  
ADD S-9

POSITIVE CONTROL

TA 100  
AF 2 UC  
ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL

TA 100

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 100

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 100

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 100

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 1538

AF 2 UG

ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 100

MNNG 2 UG

DO NOT ADD S-9

**Figure 8. Sample Label Texts for the Positive Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

POSITIVE CONTROL

TA 1535

MNNG 20 UG

DO NOT ADD S-9

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9)**

NEGATIVE CONTROL

TA 18

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 22

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 28

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 28

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 28

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 28

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 100

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 100

DO NOT ADD S-9

START RUN

Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUED

NEGATIVE CONTROL  
TA 100  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 100  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 100  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 100  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 1535  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 1535  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 1535  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 1535  
DO NOT ADD S-9  
START RUN

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

NEGATIVE CONTROL

TA 1535  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1535  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

NEGATIVE CONTROL

TA 1537  
DO NOT ADD S-9  
START RUN

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 153P

DO NOT ADD S-9

START RUN

NEGATIVE CONTROL

TA 9P

ADD S-9

START RUN

NEGATIVE CONTROL

TA 9P

ADD S-9

START RUN

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

NEGATIVE CONTROL

TA 23

ADD S-9

START RUN

NEGATIVE CONTROL

TA 100

ADD S-9

START RUN

**Figure 9. Sample Label Texts for the Negative Control  
Responses of Microbial Strains  
(With and Without S-9), CONTINUED**

NEGATIVE CONTROL  
TA 100  
ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 100  
ADD S-9  
START RUN

NEGATIVE CONTROL  
TA 1535  
ADD S-9  
START RUN

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUE**

NEGATIVE CONTROL

TA 1527

ADD S-9

START RUN

NEGATIVE CONTROL

TA 1528

ADD S-9

START RUN

**Figure 9. Sample Label Texts for the Negative Control Responses of Microbial Strains (With and Without S-9) CONTINUED**

NEGATIVE CONTROL  
TA 1538  
ADD S-9  
START RUN

**Figure 10. Sample Label Texts for Dilutional Control Check for a Test Compound**

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
DO NOT ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
DO NOT ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
DO NOT ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
DO NOT ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
ADD S-9

DILUTIONAL CONTROL  
COMPOUND: CMPND #1  
ADD NO ORGANISMS  
ADD S-9

**Figure 11. Sample Label Texts for a Test Compound  
(With and Without S-9)**

TEST SUBSTANCE  
TA 1533  
COMPOUND: CMPND #1  
DILUTION: ?  
DO NOT ADD S-9

TEST SURSTANCE  
TA 1538  
COMPOUND: CMPND #1  
DILUTION: 2  
DO NOT ADD S-9

TEST SUBSTANCE  
TA 1538  
COMPOUND: CMPND #1  
DILUTION: 2  
DO NOT ADD S-9

TEST SUBSTANCE  
TA 1538  
COMPOUND: CMPND #1  
DILUTION: ?  
DO NOT ADD S-9

TEST SUBSTANCE  
TA 1538  
COMPOUND: CMPND #1  
DILUTION: 2  
DO NOT ADD S-9

TEST SUBSTANCE  
TA 1538  
COMPOUND: CMPND #1  
DILUTION: 2  
DO NOT ADD S-9

**Figure 11. Sample Label Texts for a Test Compound  
(With and Without S-9)  
CONTINUED**

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

TEST SUBSTANCE

TA 1538

COMPOUND: CMPND #2

DILUTION: 2

ADD S-9

END

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